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THE METHOD OF SPREADING OF THE OLIVE KNOT DISEASE

WM. T. HORNE, WM. B. PARKER, AND LYMAN L. DAINES

(WITH PLATE VI)

This paper is a preliminary report on studies carried out at Fairoaks, Sacramento Co., California, supported at first by personal subscriptions of the orchardists of the locality and later under a special appropriation by the legislature for the study of plant diseases in northern California.

The etiology of the olive knot, also known as tubercle disease, tuberculosis, etc., has been very thoroughly investigated by Dr. Erwin F. Smith and the characteristics of the causative organism well set forth in 1908[1]. A preliminary report was made earlier by E. F. Smith and J. B. Rorer in a paper read before the American Association for the Advancement of Science, an abstract of which was published in 1904.[2] The disease was reported in California by Professor Frederick T. Bioletti in 1898.[3] At that time proven remedies were wanting and it was not known how serious the trouble might become.

Dr. Smith's work leaves little of an essential nature excepting the question of the method of distribution of the causative organism and the working out of a specific set of directions for the eradication or control of the disease. Professor E. B. Babcock, assisted by Mr. Wm. B. Parker, undertook the study of these problems for the relief of the promising young olive industry at Fairoaks, working mostly during the summer. A large amount of valuable data was collected and experiments made which will be described in the finished bulletin on the subject. The work done in endeavoring to fix on some insect the responsibility for carrying the specific bacteria from tree to tree gave negative results, and, thereafter, effort along this line was confined to keeping a lookout for clues.

[1] U. S. Dept. Agr., Bur. Pl. Ind., Bul. 131, Part IV. 1908.

[2] Science, N. S., 19 : 416. 1904.

[3] Univ. Cal. Agr. Exp. Sta. Bul. 120. 1898.

In 1909 the senior author of this article took Professor Babcock's place in the investigation. When visiting the groves at Fairoaks with Mr. Parker in October the trees were wet from rain of the previous day and night. On examining the vigorous knots, some were found to have an opalescent slime in the cracks and depressions. This slime had been seen before by Mr. Parker. It was now taken as the clue for solving the problem of the distribution of the organism. It became necessary to prove that the slime contains the specific organisms in considerable number and to find the way by which they penetrate into the tree to cause new infection.

In June, 1908, Mr. Parker had introduced bouillon into a crack in a vigorous knot. After stirring it about a little, taking care not to break the tissue, some of this bouillon was put into sterile bouillon which was then incubated. Inoculations from this culture produced typical knots. Needle prick inoculations directly from the slime were now made into vigorous twigs, but these were unfortunately lost before the next summer. Mr. Parker left this study for entomology, and laboratory work with the organism was not successfully carried out until Mr. Daines undertook it in 1910. The specific organism was then isolated from growing knots, studied in various media, and compared with pure cultures kindly sent by Dr. Erwin F. Smith. Tree inoculations were also made from various different pure cultures of the organism identified from culture characters.

Owing to the infrequency of rains during the winter of 1910–11, observations on the natural production of slime following rains were not made, but the causative organism has been repeatedly isolated from slime which was caused to exude from the knots by moistening and keeping them wrapped, or in a moist chamber. Very young vigorous knots will produce slime, and poured plates from these give almost exclusively colonies of *Bacterium Savastanoi*. Some of these pure cultures have been inoculated by needle pricks into living olive trees and the characteristic knots produced. Many direct inoculations into living bark from the artificially produced slime, each by a series of needle pricks, have given rise to the characteristic knots. Only one of all such inoculations has failed to produce the knot, although not all of the individual needle pricks succeed in cases where the twigs are not vigorous.

The foregoing evidence seems sufficient to show that the bacteria of olive knot, *Bacterium Savastanoi* E. F. Smith, regularly escape from the knot by exudation after rain.

The work of Professor Babcock and Mr. Parker had shown that practically no new knots appear except during the early summer.

This is readily explained by the fact that during summer no rain falls in the California valleys, and, since the bacteria are sensitive to sunlight, those on exposed surfaces are quickly killed by the drying and brilliant sunshine. On the other hand, the winter is cool and the knots may be wet for days in succession, offering abundant opportunity for exudation and distribution of the bacteria by washing and spattering. Birds and a limited number of insects are active in the olive orchards throughout the winter and their feet must distribute the bacterial slime with great efficiency. The slowness with which the disease spreads from tree to tree and orchard to orchard seems surprising in view of the facts, but the erratic manner of spreading corresponds with what would be anticipated.

We now come to the question, how do the bacteria make their way into the living cortex where they can begin their parasitic life? The search for piercing or gnawing insects having proved futile, and since there seemed to be no mark or scar to show where any insect had been at work, the theory was adopted that in nature they are not ordinarily introduced into the living tissue by a violent outside agent. By far the largest number of knots appear on leaf scars or wound calluses. Any roughened surface where there is active growth below, especially if the situation is such that growth tends to expand the surface bark strongly, is very liable to infection. Occasionally, a well-formed knot is found on a smooth limb two or three inches in diameter, where not the slightest trace of wound or injury can be detected. Most of the leaf-fall is in June when natural infection of the fresh leaf scars is impossible in the California valleys. Infection in wound calluses also evidently takes place long after the wounds have been made.

Early in these studies the theory was formed that natural infection takes place by the washing down of bacteria from slime into minute natural crevices in the bark, that here in these crevices the bacteria grow and force their way into the living cortex below. If the above theory is true, an infusion of the specific bacteria sprayed on the living bark should produce the typical knots. The first experiments in this line were made on a small scale and no knots were formed. It would not be reasonable to expect all such inoculations to succeed, otherwise, in nature, no part of an infected tree would escape. The number of experiments made in this way has not been large, but the typical knot formation has been secured on a tree not naturally infected, where the bark had been wet with an infusion of the specific organism. The trunk was wet on the day following the inoculation and covered with paper to check drying of the surface. At the time of writing, Mr. Daines has just made platings from one of these knots and shown it to

contain practically a pure culture of *B. Savastanoi*. Experiments are being continued with the hope of thoroughly confirming this result.

From these data it seems permissible to conclude that infection can take place through minute natural crevices in the bark in which the bacteria are protected from drying, while they grow and penetrate into the living cortex. In view of the lack of any other known method of introduction of the organisms in nature, we may presume that this is the usual one. The infected trees must be covered during the rainy weather of winter with an almost continuous coating of the specific bacteria. Beating or raking the trees in picking oil olives, or any other wounding, must bring the bacteria into direct contact with the living inner bark and favor infection.

Remedial measures are being worked out on the theory here suggested. Mr. Parker has already shown that the disease can be suppressed by pruning out the affected twigs and cutting the knots off the trunks. The tree becomes reinfected, however, in proximity to other infected trees. We believe that eradication is practicable but the suppression of knots on trunks has offered considerable difficulty owing to the fact that the bacterial pockets are sometimes considerably extended, and knots reappear where they have been cut off and the spot disinfected. Much thoroughness and considerable persistence will be necessary to eradicate the trouble from an orchard. Sprays of lime-sulphur and bordeaux mixture for preventing new infections are being tried.

The character of the tree will have considerable bearing on the problem. The Mission olive, which has comparatively smooth bark on trunk and limbs and an open habit of growth is less affected than the Manzanillo, which does not have so smooth and open a growth. The Nevadillo blanco, which has the trunk covered with natural swellings or uvuoli, is very susceptible. Fortunately the Mission olive is the most generally prized.

To recapitulate, our conception of the actual method of transference, in California, of the organism causing olive knot or tubercle disease is as follows :—

Under the influence of continual wetting of the knots by rain, an opalescent slime exudes from them, usually from cracks. This slime contains the specific organism, *Bacterium Savastanoi*, in great abundance. Dispersion of the bacteria contained in the slime must follow by washing and spattering by rain and in various purely accidental ways. We are convinced that infection takes place through natural fissures in the dead bark where the bacteria are kept moist while they grow through the tissues into the living cortex. This conclusion

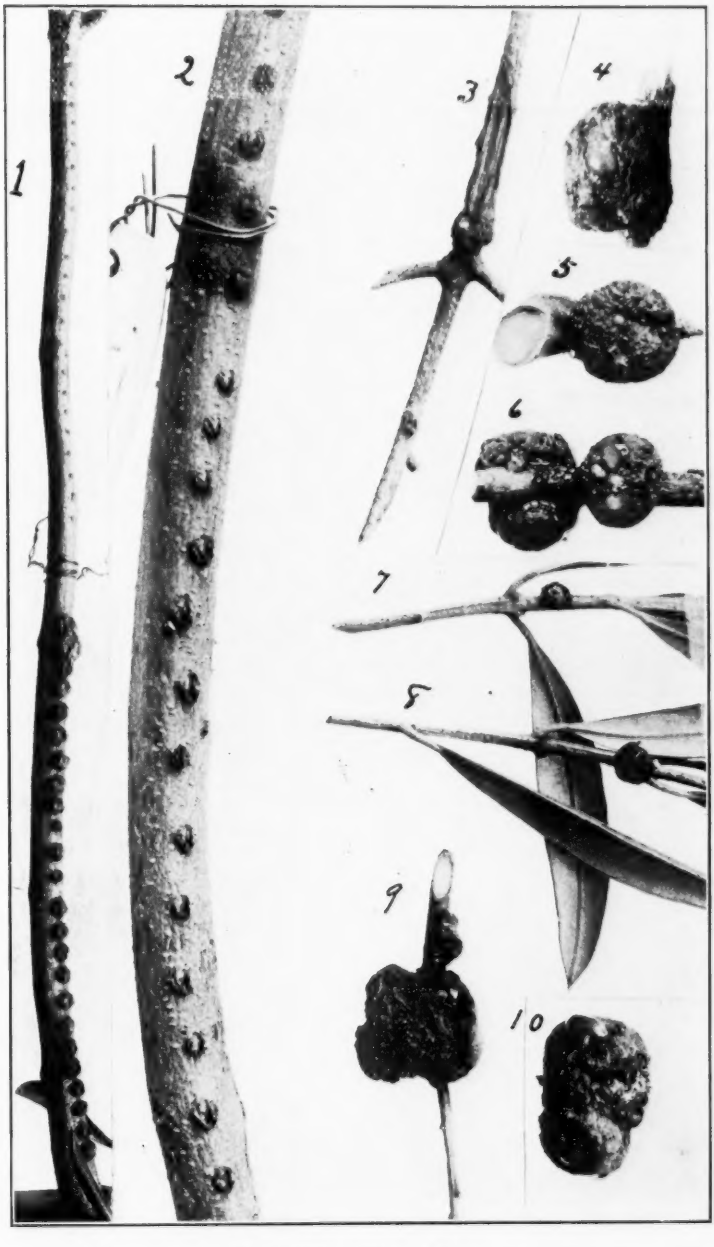

Plate VI. Olive Knot

is based on the following things: (1) the lack of evidence that in nature the bacteria are dependent on any puncturing agent to introduce them into the living cortex; (2) the fact that infection takes place most frequently on healed wounds, *i. e.*, on wound calluses; (3) that usually the infection is not introduced when the wound is made; (4) the success of a few inoculations on uninjured bark.

The generous and hearty co-operation of the citizens of Fairoaks should be acknowledged, and especially the enthusiastic and constant support and assistance of Professor Elmore Chase, Deputy Commissioner of Horticulture for Sacramento County.

Acknowledgments are also due to Dr. Erwin F. Smith for material and suggestions, and to Professor E. B. Babcock, Division of Agricultural Education, University of California, for his continued interest and assistance in the work.

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EXPLANATION OF PLATE.

PLATE VI.

FIG. 1. Twig inoculated with pure culture of *Bacterium Savastanoi* Erwin F. Smith on April 22, 1911, and cut in September, 1911. Control punctures with sterile needle are shown above. ½ natural size. This is a typical case of inoculation in an infected tree after rains cease. Inoculations made in infected trees before winter rains are over show some infections in the control punctures.

FIG. 2. Twig inoculated with pure culture, April 22, cut June 24, and kept moist for several days. Natural size. Slime showed in practically every knot and is visible in the photograph in Nos. 1, 3, 5, 6, and 9 from the bottom. Platings from this slime gave almost exclusively colonies of *B. Savastanoi*, and direct prick inoculations from the slime produced the knots in living twigs.

FIGS. 3, 7, and 8. Typical new knots from natural infection, cut June 24, 1911. Natural size. These have produced typical white slime in moist chambers.

FIGS. 4, 5, 6, and 10. Vigorous knots cut in September and kept wet for several days in a moist chamber. Natural size. Such slime is not always white opalescent and may contain other organisms than *B. Savastanoi*.

FIG. 9. Old knot cut June 24 and kept wet in moist chamber. Natural size.

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A PRACTICAL AND RELIABLE APPARATUS FOR CULTURE WORK AT LOW TEMPERATURES

N. J. GIDDINGS

(WITH PLATE VII)

The author has often felt the serious need of a good outfit for culture work at low temperatures and has learned of similar desires from many others. There are several types of such apparatus on the market, but none of them seems to be satisfactory. In some places and for certain work a satisfactory temperature may be maintained by connecting an incubator with the water supply so that there is a continuous flow of water through it. Those who have used such a cold incubator are familiar with its many faults and they need not be discussed at this time. The large incubators with compartments and an ice box in one end, are very useful for some kinds of work but are quite expensive, cumbersome, and unsatisfactory in many ways.

Some little time during the last two years has been spent in trying to devise a low temperature incubator which would at least overcome many of the objectionable features in those mentioned above. As a final result, the apparatus shown in plate VII was worked out and is giving splendid satisfaction.

The six essentials of this outfit consist of the incubator, an electric thermo-regulator, an instrument to make and break an electric lighting circuit, a small electric motor, a rotary pump, an ice box containing a coil of copper pipe through which the water surrounding the incubator chamber is forced to circulate whenever the temperature becomes too high, and a perforated copper pipe set in the water chamber of the incubator so as to secure uniform distribution of the circulating water.

The incubator is a large one secured from the Bausch & Lomb Optical Co., for use with gas. The electric thermo-regulator was secured from the above firm, and is the same as they use in their electric incubators. The instrument which is used to make and break the electric lighting current is patterned after one designed by Prof. B. H. Hite, of this Experiment Station, and he has used such a contact-maker for various kinds of incubation work during the past few years. It has given very satisfactory service, but a solenoid switch would undoubtedly be better for such work. Considerable might be said concerning the advantages and disadvantages of the two instruments but we will mention only the fact that the solenoid switch costs

about five times as much as the apparatus in use on our incubator. We will gladly give a detailed description of this instrument to any one requesting it.

The electric motor is a one-sixth H. P. Emerson. It starts free, *i.e.* does not run the pump till it has attained some speed. This is a very good feature, but not essential in so powerful a motor on this work. A 12-inch fan motor easily handles the pump, but will not always start with a load and so would not be safe unless fitted with some kind of an automatic clutch so that it would start free.

The pump is of the rotary type known as a "gear pump", and was secured from the Lipman Mfg. Co., Beloit, Wis.

The ice box (see Figure 1) is well made, having double walls with two layers of building paper and a dead air space between them. It occupies all the available space in the cabinet not required by the motor, pump, batteries and contact-maker. The inside dimensions of the ice box are; length 22 inches, width 18 inches, height 23½ inches. It contains one layer of pipe resting on the bottom and another layer separated from the first by some blocks of wood 1½ inches thick. With this amount of pipe there is still room for 150 pounds of ice without crowding. The drain pipe opening is in the back of the ice box and is ½ inch in diameter, and its center is 8 inches from the bottom. By this arrangement it is possible, if one desires to work with very low temperatures, to put in 2 more layers of pipe and still have them submerged in cold water. The height from the bottom to the door opening is 9⅞ inches, being 2 inches above ordinary cold water level, so that there is little danger of splashing out water when putting in ice. The pipe used in the coil is ¼ inch copper and known as iron pipe size. All fittings are of brass and, in place of return bends, ells and street ells were used for connecting the pieces of pipe. The latter were used because they were cheaper and easier to procure.

In one end of the incubator, near the top and back, a copper pipe is set into the water chamber (see Fig. 3). This pipe extends the full length of the chamber, is soldered in and contains 16 one-sixteenth inch holes, half of them opening toward the front of the incubator and half toward the back. The cold water forced into this pipe from the pump is distributed around the top, back and sides of the incubator, and a good, even temperature is maintained.

The action of the apparatus is as follows: Whenever the temperature gets too high, the regulator makes a contact which completes a battery circuit. The battery current acts upon the contact maker to complete the electric lighting circuit. The motor is thus thrown on and starts the pump, which draws cold water from the coil in the ice box and

forces it into the top of the incubator through the distributing pipe, while the warmer water is drawn into the coils from the base of the incubator. When the temperature has been sufficiently lowered the regulator makes another contact on its opposite side, again completing the battery circuit and operating to break the electric light circuit.

My recommendation to anyone desiring an outfit of this kind would be to purchase a Bausch & Lomb electric incubator without electric stove or base, a free starting motor of one-eighth H. P., a ¼ inch brass rotary pump, and sufficient ¼ inch iron-pipe-size copper or brass pipe, and brass fittings for the coils and connections to water chamber of incubator. A cabinet base having ice box and space for motor, pump, and solenoid switch could be made by any good carpenter, and a plumber can put in the copper lining and the pipe connections.

The total cost of the outfit recommended above, exclusive of incubator, should not exceed $80. This estimate allows $25 for motor, $3.50 for pump, $26 for cabinet, $15 for pipe and fittings, and $10.50 for plumbing. By purchasing the electric incubator without stove or base there should be a saving of $10 to $25 thus materially reducing the actual extra cost of the outfit.

In case one desired to use a smaller incubator the ice box could be made to include all the space in the cabinet, or it could be made to stand beside the incubator.

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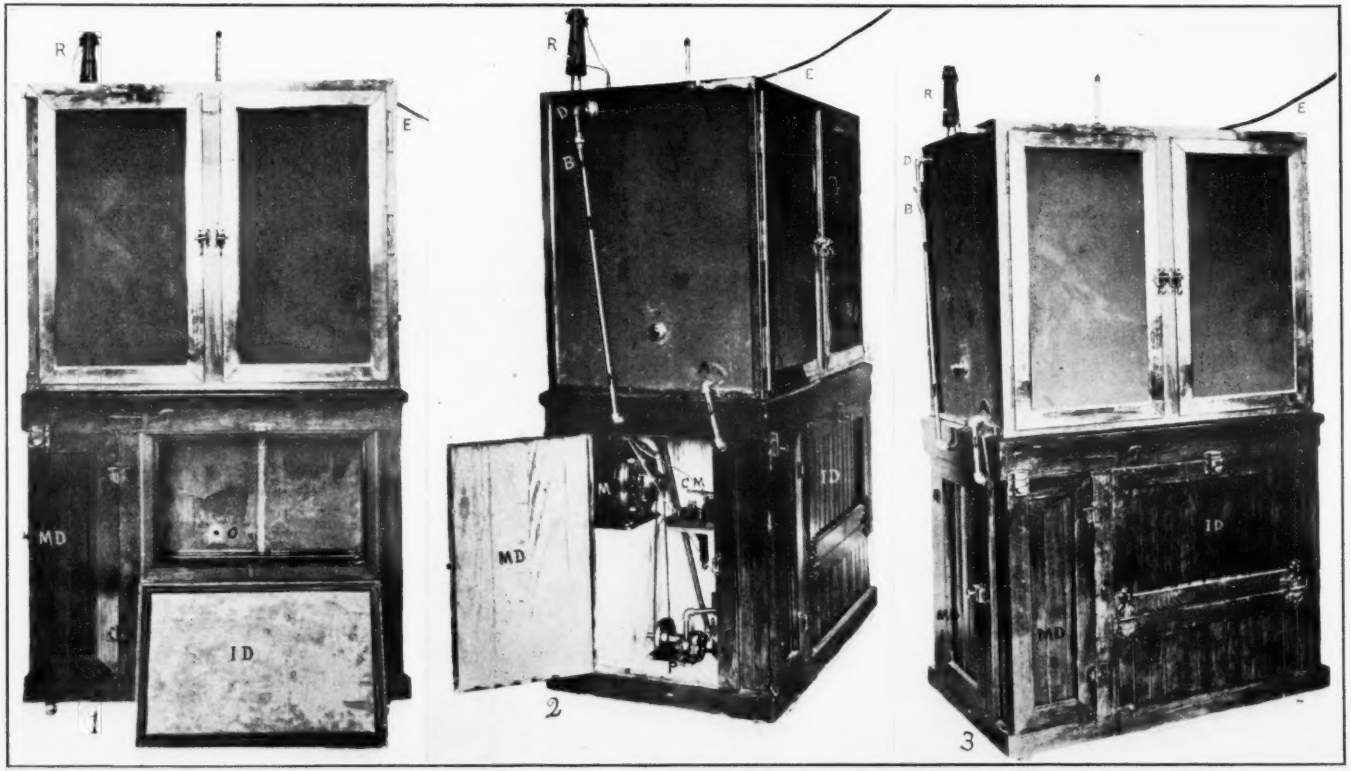

PLATE VII—**Apparatus for Culture Work at Low Temperatures**

1. Front view with ice box door open. Camera is looking down into ice box, in order to show drain pipe more clearly. *A.* Pipe leading water from water chamber of incubator to coil in ice box. The drain cock for incubator was formerly attached where upper ell is seen on this pipe. *B.* Pipe carrying cold water from pump to distributing pipe. *D.* Outer end of distributing pipe. *E.* Wire leading from electric light socket to motor and contact-maker. *M.* Electric motor. *O.* Drain pipe opening in ice box. *P.* Rotary pump. *R.* Thermo-regulator. *CM.* Contact-maker. *ID.* Door of ice box. *MD.* Doors of compartment containing motor, pump, etc.

2. End view with front and end doors of motor compartment open.

3. Front view of incubator and cabinet complete.

THE CAUSE OF STEM-END ROT OF CITRUS FRUITS (PHOMOPSIS CITRI N. SP.)

H. S. FAWCETT

(WITH PLATES VIII AND IX)

In November, 1909, an investigation as to the cause of a new kind of decay of citrus fruits, called stem-end rot, showed that a particular fungus was constantly present, which, on being isolated and placed in contact with the base of sound picked fruit, would reproduce the same kind of rotting.[1]

In the grove, stem-end rot first appears in August or September on immature oranges or grape-fruit as a dark brown, reddish-brown, or black discoloration about the base of the fruit. More often than not, the fruits drop off before any discoloration begins (Plate VIII, Fig. 1). Records of trees in four localities where this disease was present showed that from 15 to 32 per cent of the fruit fell off between October, 1910, and February, 1911. Of these dropped fruits, 35 to 85 per cent showed symptoms of stem-end rot soon after falling. When the disease attacks the mature fruit, a circular patch at the base becomes soft without discoloration. As the softened area enlarges, covering one-third to one-half of the surface of the fruit, the yellow or orange color of the rind changes through dull brown to dark coffee color. On opening the fruit the decay will be found to have proceeded most rapidly along the fibrous core in the center and along the white inner part of the peel. This rotting may cause much destruction of packed fruit while in transit and after arrival on the market. Shipping tests of carefully picked and packed fruit (from the same trees from which the records of dropping were taken) showed an amount of stem-end rot from zero up to 10 per cent immediately on arrival at Washington; 0.6 to 30 per cent one week after arrival; 8 to 52 per cent two weeks after; and 18 to 68 per cent three weeks after.[2]

THE FUNGUS

The fungus was readily isolated from the interior of partially decayed oranges, both by putting minute pieces of these into glucose agar in petri dishes, and by dropping bits into sterilized orange or

[1] Fla. State Hort. Soc. Proc. 1910: 73-78.

[2] This test was made in cooperation with the Fruit Investigation Division of the Bureau of Plant Industry, U. S. Dept. Agr.

prune juice. Other fungi or bacteria were not of frequent occurrence in this stem-end rot material. The same fungus was isolated from more than fifty different specimens of rotting citrus fruits and diseased citrus branches from more than twenty-five localities in Florida. It was distinguished, in either neutral or acid media, from most of the common citrus-inhabiting fungi, by its chalky growth remaining pure white. In alkaline media the growth was slower and the mycelium more delicate. (Plate IX, Fig. 3, A.) At a temperature of 20-25° C, on most of the ordinary media, large mycelial growths were formed in two or three days. No spores of any kind were found on either agar plate or tube cultures, and seldom in prune juice cultures. Not until November, 1910, a year after the investigation began, was it discovered that pycnidia and spores could be produced in three or four weeks by transferring the fungus to sterile live orange wood. The pycnidia were embedded in a white mat of surface mycelium, and from these the spores oozed out in round light-yellow masses, or in long strings. Pycnidia were also obtained in about four weeks by inoculation of picked green oranges with either the mycelium or spores from pure cultures (Fig. 2). The pycnidia often appeared to be white or gray, but, on sectioning, their walls were found to be greenish or dark in color (Fig. 4). The spores were oval to spindle-shaped (Fig. 5). The paraphyses sometimes came streaming out with the spores, and it was thought at first that they might be another form of spore. Repeated attempts to germinate them, however, failed. C. L. Shear[1] refers to such bodies as scolecospores, while other authors consider that until they are shown to be capable of germination they may as well be called paraphyses. When grown in either dilute prune juice or orange juice, the fungus invariably produced Achlya-like branches, with protrusions of protoplasm from the ends. These masses of protoplasm usually formed many distinct balls and gave the impression of spore formation.[2] This condition of the fungus misled the author at first into thinking that it might prove to be a species of Achlya.[3]

The growth of the pycnidia of this fungus in pure cultures led to their identification upon dead twigs and dead bark on the trees and upon rotted fruits on the ground. The twigs from which the infected fruit had dropped frequently died back to a distance of 3 to 12 inches and pycnidia of this fungus grew on the dead parts. Small drops of gum oozed out near the junction of dead and live wood. Pure cultures

[1]Phytopathology 1: 116. 1911.
[2]Fla. Agr. Exp. Sta. Bul. 107. Fig. 6.
[3]Fla. State Hort. Soc. Proc. 1910.

of fungus were obtained from the interior of these twigs and proved to be the same fungus as that in the cultures from the interior of rotted fruits. Pure cultures put into cuts in the bark of branches induced slight gumming, and killed the adjacent tissue, while the pycnidia developed on the killed bark.

INOCULATION EXPERIMENTS

The details in regard to a series of inoculation experiments with mature picked fruits were given in the Annual Report of the Florida Agricultural Experiment Station for 1910 and in the Proceedings of the Florida State Horticultural Society for the same year. These experiments showed that the fungus could most easily infect the fruit through the cut stem; that it might be transmitted from diseased oranges to sound ones by contact, or by soaking them in the same water; that it might be transmitted by means of pure mycelial cultures of the fungus placed on the fruit or in the water in which the fruit was soaked; and that fruits could be infected by placing them in water with soil from under infected trees. Further experiments showed that infection could be produced by simply placing the spores on the cut stem of fruits, and then wrapping and packing as for market, without supplying moisture[6].

The growth of the fungus in young green picked oranges (both with and without injury) is shown by the following results of inoculations made by O. F. Burger in August, 1911. The fruits were carefully picked, wiped with a cloth moistened with mercuric chloride (1-1000) and the stems freshly cut across with a sharp scalpel without injuring the rind of the fruit. The inoculated fruits, with uninoculated fruits as checks, were wrapped and set aside in open aquarium jars without adding moisture. Slight injuries, such as punctures into the end of the stem or a scratch on the peel, caused a more rapid decay, as would be expected, but a large percentage of the inoculated fruits that were not injured also rotted.

INOCULATION OF GREEN PICKED FRUIT

Inoculated Aug. 3, 1911	Number of fruit	Number showing stem-end rot 8 days	16 days	32 days
Checks, not injured, not inoculated	15	0	0	0
Checks, stem punctured, not inoculated	11	0	1	1
Checks, peel scratched, not inoculated	4	0	0	1
Spores put on end of stem	8	0	1	5
Mycelium put on end of stem	4	1	2	4
Spores put on punctured end of stem	5	0	2	5
Mycelium put on punctured end of stem	5	0	2	5
Spores put on scratched peel	4	0	0	4
Mycelium put on scratched peel	4	0	4	4

[6]Fla. Agr. Exp. Sta. Bul. 107. 1911.

Inoculated Aug. 8, 1911	Number fruit	11 days	15 days	30 days
Checks, not injured, not inoculated	12	1	1	1
Checks, end of stem punctured, not inoculated	12	0	1	1
Checks, peel scratched not inoculated	12	0	0	0
Spores put on end of stem	10	1	6	9
Spores put on punctured end of stem	10	2	4	9
Spores put on scratched peel	8	4	7	7

Several green oranges on the trees in the greenhouse were inoculated in September, 1910, by placing mycelium under the calyx. No development of the disease was observed. Other inoculations were made in May, 1911, both by placing spores under the calyx, and by puncturing the rind slightly with a needle and putting on spores. Raised corky warts were formed at the punctured points but the fruits were not otherwise injured up to September, 1911, when the last observation was made. In many inoculations of growing branches and twigs, however, by insertion of mycelium or spores into cuts made through the bark, with checks cut for comparison, gumming was induced with more or less killing of adjacent tissue. The check cuts (not inoculated) healed without gumming. When small twigs were inoculated, some of these were killed back, after which the fungus was obtained at considerable distances from the inoculated points. So far, however, all inoculations made by placing fungus mycelium or spores on the uninjured bark, or on the rind of green fruit on growing orange trees, have failed to show any effects.

IDENTITY OF THE FUNGUS

A study of the pycnidia of this fungus indicated that it might possibly belong to the genus Zythia. Specimens on orange bark were sent to Mrs. Flora W. Patterson, Mycologist, Bureau of Plant Industry, Washington, D. C., who kindly gave most careful study to the fungus and reported as follows :

"According to the latest investigations, it must be classed as a Phomopsis. Before reaching this conclusion, after long study of your specimens and slides, and the slides prepared here, descriptions of all species of Zythia have been read and slides prepared from several sets of exsiccati. The only one nearly approaching your organism, as found in your small specimen submitted in July of this year, was *Zythia versoniana*, Sacc. (Phomopsis) Saccardo Sylloge **3**, p. 614. In none of the slides nor material submitted in October, 1911, has the color of the pycnidia been such as would warrant the classification as among the Nectrioideae. The only bright colors showing on this material are connected with the bark, especially when it is elevated

FIG. 1.

FIG. 2.

PLATE VIII—**Rot of Citrus Fruits**

Fig. 1. Dropping of Grapefruit from stem-end rot in October.

Fig. 2. Pycnidial pustules produced in 35 days by inoculation of a green immature orange with spores from a culture on sterilized orange wood. x 1½. Photograph by J. B. Griffith.

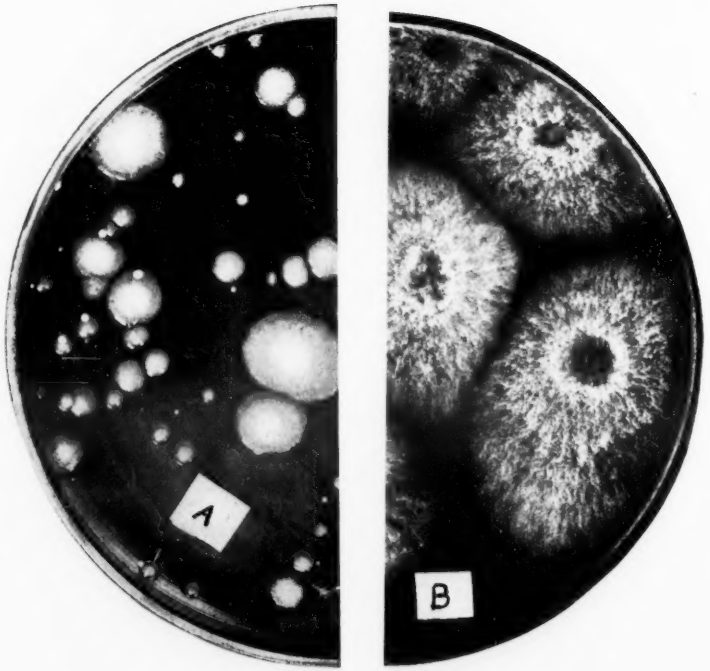

FIG. 3.

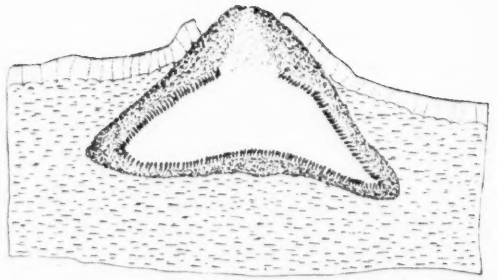

FIG. 4.

FIG. 5.

FIG. 6.

PLATE IX—**Rot of Citrus Fruits.**

Fig. 3. Growth of the fungus on standard peptonized beef agar. A. With 3 per cent of normal alkali. B. With 8 per cent normal acid. Photograph by O. F. Burger.

Fig. 4. Diagrammatical section of a pycnidium breaking through the epidermis of the bark of a small orange branch. x 60.

Fig. 5. Portion of the wall of a pycnidium showing arrangement of spores and paraphyses. x 750.

Fig. 6. Spores germinating in dilute prune juice after 24 hours. x 750.

previous to bursting. Under these elevations in many instances there is no pycnidium, and when one is present it is dark green and of a parenchymatous texture, neither waxy nor fleshy. Various mycologists have claimed a connection between Phomopsis and Diaporthe, and many species heretofore described as Sphaeronema, Phoma, Fusicoccum, Myxosporium, Libertella, and others, may perhaps be pycnidial stages of Diaporthe. In the recent comprehensive work by H. Diedicke, 'Die Gattung Phomopsis', in Annales Mycologici, Vol. IX, February, 1911, no species is reported on any citrus host."

A search of the literature has been made both by Mrs. Patterson and the writer without finding any species of Phomopsis or Diaporthe reported on any citrus host. The technical description is as follows:

Phomopsis citri, n. sp. Pycnidia scattered, ovoid, parenchymatous, green or dark colored, 200-450 microns diameter, erumpent, thick-walled, top easily broken away leaving spores exposed. Ostiole 35-45 microns in diameter. Spores ovate or fusiform, often flattened on one side, hyaline, often 1-3 guttulate, 5-9 x 2.5-4 microns, mostly 6-8 x 3. Basidia 1.5 x 12-15 microns. Paraphyses 20-30 x 0.75-1.5, easily detached, often curved at one end. On dead branches and decayed fruits of *Citrus aurantium*, *C. decumana*, and *C. nobilis*, in Florida, U. S. A.

Pycnidiis sparsis, ovoidiis parenchymaticiis, viridis vel atro-viridis, 200-450μ diam.; ostiolis 35-45μ diam.; sporulis ovatiis vel fusoideis, plerumque complano-inaequilateris, hyalinis, 5-9 x 2.5-4μ, majoribus 6-8 x 3μ, saepe 1-3 guttatis; basidiis 12-15 x 1.5μ; paraphysibus bacillaribus 20-30 x .75-1.5μ, copiosis, fugacibus, saepe apice curvis.

Hab. in ramis emortuis et fructibus putridis *Citri aurantii*, *C. decumanae* et *C. nobilis*, Florida, Amer. bor.

The details and results of a preliminary series of experiments for the control of stem-end rot are published in Bulletin 107 of the Florida Agricultural Experiment Station, and in the Annual Report of the same Station for 1911.

GAINESVILLE, FLORIDA

WOOD ROTS OF THE HARDY CATALPA

NEIL E. STEVENS

(WITH PLATE X)

During an investigation of the fungus diseases of the hardy catalpa on the various Kansas plantations the writer has made several observations of fungi growing on dead catalpa wood. While it seems almost superfluous to advance proofs that the wood of a given species, even a very resistant species, will rot, and that the rot is due to fungi, it may be worth while to call attention briefly to these forms, for the growth of any fungus upon so resistant a wood as the catalpa must be of more than usual interest. These observations seem especially worthy of note since up to the present time there is no record of fungi which will grow upon dead catalpa.

Bulletin No. 37 of the Bureau of Forestry is the only publication which has appeared dealing at any length with wood rots of the catalpa. Papers dealing with catalpa which have appeared since that time have drawn their information as to wood rots chiefly from this source. In this paper, (5 : 49) it is stated that "none of the ordinary saprophytic wood-destroying fungi grow in catalpa wood; in fact no fungus has yet been found which will grow in the dead timber". The fact that catalpa does not last forever is attributed to the "alternate wetting and drying of the wood fibres, causing expansion and contraction". This is in itself sufficiently remarkable, but the writer further states, (5 : 54) that *Polystictus versicolor*, while capable of rapid growth and destruction of the wood of a living catalpa tree, stops its growth when the tree is cut.

As is well known, *Polystictus versicolor* is a very common and very destructive saprophyte on the wood of many species of deciduous trees. That it should become parasitic on a given species of wood is not in itself remarkable, for other wood rotting saprophytes are known to become parasitic on certain favorable hosts. But if such a typical saprophyte after growing vigorously as a parasite on a tree should prove not only incapable of infecting dead wood of that species but unable even to continue growth in an already infected log after the tree was cut it would be indeed, "a curious case of close adaptation".

The statements quoted above seem to be based on two points; the unquestioned durability of catalpa, and the fact that no sporophores of a wood rotting fungus were found on catalpa timber. It is, however, quite unwarrantable to conclude that because a piece of wood bears no sporophores it therefore contains no wood rotting fungi. Such a con-

clusion involves the entirely erroneous assumption that a fungus will fruit wherever it will grow. Little is, of course, known of the physiological conditions governing the production of fruiting bodies by wood rotting fungi, but it has several times been demonstrated that such a fungus may make vigorous vegetative growth and even destroy a considerable portion of the wood upon which it grows without producing sporophores. In another paper (4 : 26) von Schrenk describes a fungus which damages a very large percentage of the cypress trees wherever this species grows. No fruiting organs of this fungus have, however, been found (4 : 53). It is of course possible that the fungus, though unquestionably a wood rotting species, differs considerably from most timber-destroying fungi.

There is at hand, however, experimental evidence that typical wood saprophytes may make a very rapid growth without being able to fruit. In growing *Lenzites sepiaria* in pure culture Spaulding was unable to obtain normal sporophores (3 : 19) although the decay due to the fungus (3 : 30) under these conditions was apparently identical with that which "constantly accompanies the fruiting bodies in field and forest". Rumbold (2) reports like results with pure cultures of the same species. Bayliss (1) experimented with *Polystictus versicolor* in pure culture and was unable to obtain sporophores until the cultures were taken into the open and placed on branches. This observer reports that color and pore formation depended greatly on light, no pores developing in the dark. The writer has been experimenting with this species grown in pure culture on wood of *Liriodendron tulipifera* for over two years and has as yet been unable to obtain fruiting bodies. That the failure to produce sporophores was not due to lack of growth is proved by the fact that in six months after inoculation the wood had lost over half its weight and in less than a year was so far decayed that pieces half an inch in diameter were easily crushed between the fingers. Whatever these results may prove as to the conditions governing sporophore production in this species they at least prove that *Polystictus versicolor*, like other saprophytic fungi, may grow upon and destroy wood without producing sporophores.

Moreover the writer has several times found fresh fruiting bodies of common wood rotting fungi on dead catalpa wood. In the Farlington plantation at Farlington, Kansas, the writer observed on sprouts which had been cut for three years several sporophores of *Schizophyllum commune* and many of *Polystictus versicolor*. These sprouts varied considerably in size, but most were under four inches. The fresh condition of the sporophores, as well as their orientation, proved that they had developed after the sprouts were cut. In the same plantation a

catalpa log was found which had old sporophores of *Polystictus versicolor* on both cut ends.

In the Munger plantation at Eureka, Kansas, where there were a large number of sprouts lying on the ground, some of which had been cut for three years, and others for two, fresh sporophores of both these species were found, as well as one sporophore of *Polyporus adustus*. In this place *S. commune* seemed to be more common than *P. versicolor*. Fresh sporophores of *Stereum albobadium*[1] have been found by the writer near Manhattan, Kansas, on catalpa wood which had apparently been cut for some time, and at Soldier, Kansas, on catalpa bean poles which had been cut four years.

More striking instances of the growth of fungi on dead catalpa were found at the Hunnewell plantation near Farlington. This plantation has been continuously under one management since it was started, so that fairly accurate records are easily obtainable. At this place is a pile of rotton posts, part of those cut when the second thinning was made in the winter of 1902. All these posts were apparently sound at the time of cutting as only sound posts were hauled out of the woods. The entire pile remained untouched until 1906, when it was sorted, the sound posts sold, and the rotten ones (a comparatively small per cent) thrown out. On one of these rotten posts the writer found in November, 1911, nine years after it was cut, several fresh sporophores of *Polystictus versicolor*, small but typical and fully matured (Fig. 1). It is interesting to note that while the entire pile of several cords consisted of well rotted posts, only one was found which bore sporophores. This observation gives some indication of the amount of decay that may occur although very few sporophores are produced.

These posts were so small that a considerable part was sapwood and so, in all probability, more subject to decay. On this plantation, however, a group of fresh sporophores of *P. versicolor* was found on the cut end of a tree trunk which had lain on the ground for five years. The entire group of sporophores covered an area about 5 x 10 inches, they were well developed and apparently normal. There can be no question as to whether the fungus was growing on the heartwood in this case, for the tree showed twenty-two annual rings and none of the sporophores were nearer the bark than the sixteenth. Sporophores of *Polyporus adustus* were also found on this log. It is, of course, possible that in both these cases the wood was infected when it was cut, though this seems improbable in the case of the log referred to, for the tree was the largest found on the plantation when it was cut

[1] The writer is indebted to Professor A. H. Graves of Yale University for the determination of the species referred to in this paper.

over, and, as the "prize tree", was examined and photographed several times. It is evident that if the fungus gained entrance to the posts while they were living trees, it did not die when they were cut, but made a very considerable growth and lived at least nine years.

Definite proof that the mycelium of *Polystictus versicolor* growing in living catalpa may continue growth after the tree is cut was found on the plantation of Mr. George Newcomb at Morrowville, Kansas. A well rotted tree was cut in June, 1911, and a portion several feet long was left lying on the ground. This portion was six inches in diameter and showed a soft and "punky" center surrounded by about an inch of comparatively sound wood. It was in fact a typical piece of well-rotted catalpa such as may be found in almost any tree which bears sporophores of *Polystictus versicolor*, and differed very little from the excellent figure given in Bureau of Forestry Bulletin 37, Plate 26, Fig. 2.

On November 30th, 1911, fresh sporophores of *Polystictus versicolor* were found on the cut end of this log and on the split surface where the tree had broken when it fell. That the fungus was alive and in an actively growing condition at this time, was proven when a portion of the end which bore the sporophores was cut off and placed in the green house. The sporophores already formed were broken off, and within two or three days showed signs of regeneration. Pore formation began to be evident in less than a week and in five weeks time completely-formed sporophores varying from an inch to an inch and a half in width were developed, not only in the place of the sporophores which were broken off but also on the freshly cut end of the log. Figure 2 shows the sporophores at the end of five weeks growth. In order to determine whether the fungus would retain its vitality for any length of time while growing as a saprophyte on catalpa this crop of sporophores was removed at the end of six weeks, and under the same conditions the development of new sporophores began almost immediately. A third crop of fully developed sporophores was removed February 20, and a fourth on April 10. Moreover, this is by no means an isolated case, as several pieces of well-rotted catalpa wood, all of which bore sporophores of *Polystictus versicolor* were taken from smaller trees and kept for two months in the dry air of the laboratory. They were then placed in the green house and without exception, began the development of new sporophores within a few days.

It would appear from the above observations that at least four typical wood-saprophytes can grow on dead catalpa and it is probable that others will be found. Moreover, as will be shown in a later paper,

three, and perhaps all, of these species seem to be capable of living to some extent as wound-parasites of catalpa. It seems probable that the greater part of the decay of catalpa wood is due to *Polystictus versicolor*. This species is by far the most destructive on living catalpa. It is the only species found thus far fruiting on heart-wood, and most of the rotten catalpa examined agreed closely in appearance with wood which bore sporophores of this species. Indeed in some cases, if this rotten wood which bears no sporophores be placed under favorable conditions sporophores of *Polystictus versicolor* will develop. A striking instance of this was recently observed by the writer. A portion of a well rotted catalpa post was found, which had been cut in 1906. At that time the post was apparently sound and when set was driven into the ground with a sledge. Three years later (1909) the post was so rotten that it broke and had to be replaced. A portion of the rotten post was, however, left hanging by the two upper wires of the fence and on this portion, when brought into the moist conditions of the greenhouse in January 1912, a typical sporophore of *Polystictus versicolor* developed in less than six weeks. That *Polystictus versicolor* should be more destructive to catalpa than any of the other species mentioned above seems probable also from its effects on many other kinds of woods. Von Schrenk and Spaulding make the following statements regarding the prevalence and destructiveness of this species (6 : 53).

"Of all the fungi which grow upon the deciduous species of woods after they are cut from the tree the most widely distributed, and in many respects the most destructive, is *Polystictus versicolor* . . . . . *Polystictus versicolor* is one of the most cosmopolitan species of fungi known. It does not seem to have any preference for any particular kind of wood, but grows with almost equal readiness, especially on the sapwood, on every broadleaf species of wood (as distinguished from coniferous wood) known. On account of its wide geographical range and its ability to grow on and destroy so many different kinds of wood, it should be regarded as the most serious of all the wood-rotting fungi which attack the dead wood of broadleaf trees. It is the fungus which destroys probably 75 per cent or more of the broadleaf species of timber used for tie purposes. Wherever broadleaf species of wood are used for fencing, for posts or poles, or for any purpose where they come in contact with the soil they are sure to be attacked sooner or later by *Polystictus versicolor*".

Polystictus versicolor is certainly much more destructive under some circumstances than is *Schizophyllum commune*, the species next most frequently found on catalpa. In the experiments referred to above the writer grew these two fungi in pure cultures on the wood of *Liriodendron tulipifera*, and the relatively greater destructiveness of *Polystictus versicolor* on this species of wood was clearly shown. Grown on

PLATE X.—**Wood Rots of Catalpa**

FIG. 1. Three sporophores of *Polystictus versicolor* which developed on a rotten catalpa post nine years after it was cut. x ⅔.

FIG. 2. Sporophores of *Polystictus versicolor* on partially decayed catalpa six months after the tree was cut. The sporophores shown developed in five weeks. x ⅔.

plugs of this wood in test tubes which were kept in a warm moist atmosphere, *Polystictus versicolor* occasioned a loss in six months of about 59% of the original weight of the wood. While in the same time, under the same conditions *Schizophyllum commune* occasioned a loss of only 37% of the original weight. This is of course only an approximate determination, but may perhaps be taken as roughly indicative of the rate of destruction of this species of wood by the two fungi.

The proof that these species of fungi are able to grow and fruit on catalpa wood does not, of course, affect the fact of the unusual durability of catalpa timber. It merely opens the way for a more intelligent study of the reasons for this extraordinary durability.

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MANHATTAN, KANSAS.

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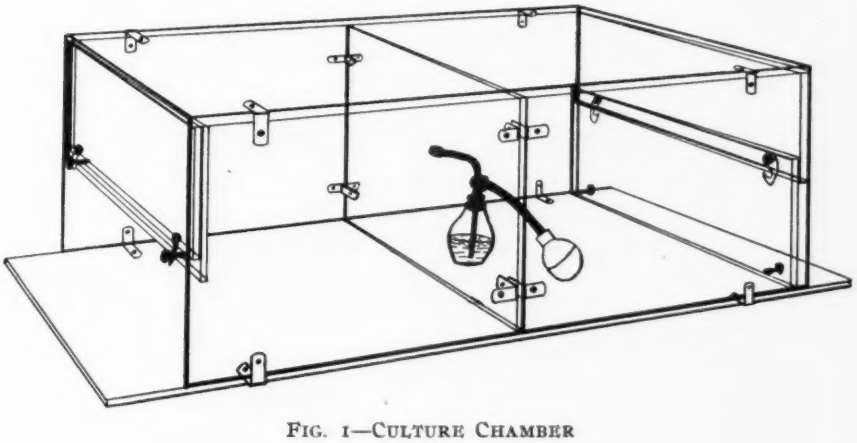

FIG. 1—CULTURE CHAMBER

A NEW FORM OF PURE CULTURE CHAMBER

E. MEAD WILCOX AND G. K. K. LINK

(WITH ONE TEXT FIGURE)

Various attempts have been made in many laboratories to construct special rooms, cages or other styles of chambers designed to overcome the delays and difficulties arising from contamination. Our experience has been that the larger rooms often provided for this purpose soon become, in spite of positive instructions to the contrary, mere catch-alls for all sorts of apparatus. In this manner the dangerous conditions of the larger laboratory room are soon duplicated on a smaller, though equally troublesome scale. The apparatus here described seems to overcome these difficulties and it is hoped its description here may prove of interest to plant pathologists.

This chamber is constructed throughout of plate glass held together by the usual nickel-plated metal parts. It is thirty-six inches long, twenty inches wide and ten inches high, divided by a transverse partition into two compartments. Each compartment has a glass door which slides vertically in a nickel-plated metal track. This door is held up by means of two clips which fall and catch beneath the knobs on the door when raised. When the door is raised there is formed an opening beneath it twenty inches wide and four inches high permitting easy access with the hands and arms.

The interior of the compartment may readily be disinfected by means of an atomizer containing either alcohol or the usual solution of corrosive sublimate. It is desirable, though not an absolute necessity, that the hands be washed in corrosive sublimate solution before beginning the work. Petri dishes containing the usual agar media have been exposed inside the cage for an hour with the door open without showing the least contamination after subsequent incubation for twenty-four hours.

An alcohol lamp may be kept in the compartment for use in sterilizing the inoculation needles though if these be kept in alcohol equally good results may be secured. By the use of such a chamber as this, one may work with a great degree of certainty that contamination will not occur—even when petri dishes and culture tubes are left uncovered for some minutes.

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DIAPORTHE, THE ASCOGENOUS FORM OF SWEET POTATO DRY ROT

L. L. HARTER AND ETHEL C. FIELD

(WITH FOUR TEXT FIGURES)

In 1890, Halsted[1] briefly called attention to the disease of sweet potatoes which was described by Ellis and Halsted as *Phoma batatae*. Little or no work has since been done on the sweet potato dry rot and Halsted did not consider the disease of much importance.

In December, 1910, specimens of sweet potatoes, showing the typical appearance of dry rot, were collected in the Washington market. An examination of this material showed that, besides the typical pycnospores of the genus Phoma, a second type of spore was present in some of the pycnidia. The latter spores were long, slender, continuous and very frequently hooked at one end, resembling in every way the spores which Diedicke[2] designates as "β" spores in the genus Phomopsis and Shear[3] calls scolecospores in the pycnidial form of *Cryptosporella viticcla*. These are apparently identical with the stylospores of Nitschke[4] in his original description of the genus Diaporthe.

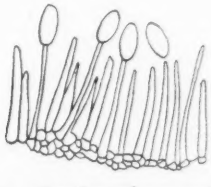

FIG. 1. Section of a pycnidium showing the pycnospores and conidiophores.

FIG. 2. Stylospores frequently found in connection with the pycnidial stage on the stem and root of the host.

Poured plates were made and from these transfers from isolated colonies were made to beef agar tubes, and, subsequently, from these to corn meal flasks. Pycnidia containing pycnospores developed in about six to ten days after the final transfers. These were left at room temperature and not examined until March 8, 1911. Out of the set of four corn meal flasks, two were found to contain mature asci.

[1]Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Exp. Station Bul. 27. 1890.

[2]Diedicke, H. Die Gattung Phomopsis. Annales Mycologici **9**: 8. 1911.

[3]Shear, C. L. The ascogenous form of the fungus causing the dead arm of the grape. Phytopathology **1**: 116. 1911.

[4]Nitschke, Th. Pyrenomycetes Germanici p. 240. 1870.

The other two flasks formed only the pycno- and stylospores, and never produced asci. It was at once suspected that this might be the ascogenous form of the dry rot organism. Poured plates were made from one of the corn meal flasks and asci isolated and kept under close observation for one or two days, or until the spores had germinated and it was evident that no foreign organisms would accompany the transfer to beef agar tubes. In this manner 25 asci were isolated at different times from three generations. Transfers were later made to corn meal flasks and in these asci were produced in four to five weeks. Pycnospores developed first in the flasks in six to ten days after inoculation, while the long, hook-shaped bodies, or stylospores, usually formed a little later.

Apparently two strains have been obtained; one which does not form perithecia in cultures (at least under ordinary conditions), and another which produces perithecia readily. From this latter strain perithecia have been readily obtained on corn meal and rolled oats

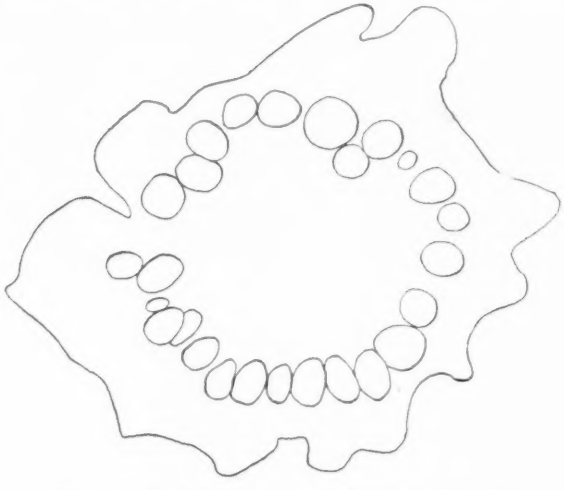

FIG. 3. Tangential section through a stroma showing the arrangement of the perithecia. From corn meal flask.

and sparingly on sweet potato cylinders. The morphology of the pycno- and stylospores of the two strains is the same when compared on various media. The ascogenous stage as yet has not been found on the host itself.

A careful study of the fungus, both in cultures and on the host, shows that the pycnidial form evidently does not belong to the genus Phoma. The pycnidia on the potato are irregularly chambered, stro-

mate and contain in many cases stylospores, which Reddick[1] in his species *Fusicoccum viticolum* regards as free paraphyses. Various attempts have been made to germinate these bodies but without success. The stylospores are also normally formed on the stem or vine of the plant.[2] The formation of a stroma and chambering of the pycnidia, however, does not occur on the stem or leaves. In cultures, the stylospores, chambering of the pycnidia and stroma are normally developed. Pycnospores and stylospores develop abundantly in the same pycnidium or either may be found alone. So far as these characters are concerned Phomopsis would best represent the imperfect form if the recent revision of the genus by Diedicke[3] is followed. If Saecardo's classifica-

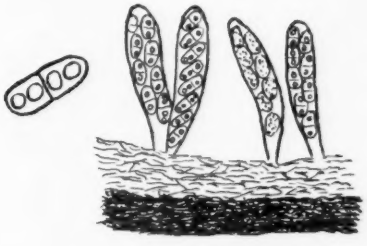

FIG. 4. Section of a part of a perithecium showing asci and spores of *Diaporthe batatatis*.

tion of this group is followed, the fungus because of the persence of a stroma would be put into either the genus Dothiorella or Fusicoccum. The writers are inclined to regard the genus Phomopsis as best representing the imperfect form.

An examination of type material of *Phoma batatae* loaned by the New York Botanical Garden shows that the two fungi are identical although no stylo-spores were found.

A full description of this disease will be given soon in a forthcoming bulletin of the United States Department of Agriculture.

The organism is described tentatively as follows:

[1] Reddick, D. Necrosis of the grape vine. Cornell University Agr. Exp. Sta. Bul. 263: 331. 1909.

[2] Inoculations have been successfully made, in both greenhouse and field, with cultures descended from the original isolations. Pycnidia containing both pycnospores and stylospores have developed on stems and roots of inoculated plants. Numerous re-isolations have been made and when compared culturally were found to be identical with the original strain.

[3] Loc. cit.

Diaporthe batatatis[1] n. sp.

1. Pycnidiis in radicibus laxe gregariis, atris, immerso-erumpentibus, ostiolatis, stromate adnatis vel immersis, irregulariter locellatiis. Pycnidiis in foliis et caulibus plerumque sub-globosis, sine stromate. Sporulis oblongo-fusoideis 6-8 x 3-5μ, continuuis, hyalinis, 2-guttulatis; basidiis filiformibus, rectis curvulisve leviter. Stylosporis filiformehamatis, curvulis rectisve raro, 16-30μ longis, continuuis; basidiis brevibus, hyalinis, continuis, subulatis; stylosporis sporidiisque in pycnidiis iisdem vel separatis. (*Phoma batatae* Ell. and Halst.). Figs. 1-2.

2. Stromatibus valsoideis, immerso-erumpentibus, extra carbonaceis, intra cinereis, cum pluribus subcylindricis rostris exsertis, 5-3 mm. longis; peritheciis in singulo stromate 9-25, subglobosis, immersis, 120-370μ latis; ascis clavato-cylindricis, aparaphysatis, sessilibus, octosporis, 23-38 x 7-12μ; sporidiis plerumque distichis, subellipticis, utrinque obtusis, hyalinis, uniseptatis, medio leviter constrictis, 2-4 guttulatis, 8-12 x 4-6μ. Figs. 3-4.

Hab. in radicibus, caulibus et foliis *Ipomoeae batatis*.

Type specimens deposited in the herbarium of the pathological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

[1] The ascogenous form is described from the character of its growth in artificial cultures.

A NEW INTERNAL STERIGMATOCYSTIS ROT OF POMEGRANATES

S. M. McMURRAN

During the summers of 1910 and 1911 a serious pomegranate disease appeared at various points in the southwestern portion of the United States. Mr. T. H. Kearney, who made observations upon this trouble in connection with his work for the Department of Agriculture, reports it at Indio, California, Sacaton, Arizona, and San Antonio, Texas; and states that it appears to be very generally distributed through that portion of the country. A grower in Georgia reports that no such disease of the fruit has been observed there, but no further data have been obtained from the southern states.

Early in November of last year a number of fruits were sent in to the office of Fruit Disease Investigations, U. S. Department of Agriculture for examination. There were external signs of decay on only one of the fruits and this consisted of a dark-brown circular spot about 2.5 cm. in diameter. None had any mechanical injuries on the rind and, but for the spot mentioned on one fruit, appeared perfectly sound and normal. They were all washed for one minute in alcoholic mercury bichlorid solution, 1-500, rinsed in sterile water, and opened under strictly aseptic conditions. Such fruits as were diseased showed a central cavity occupied by a black sporulating fungus. Except in the one spotted fruit, the fungus at the center showed no connection with the rind, being surrounded by sound tissue. Poured plates and transfers to bean pods with a sterile needle gave but one organism, *Sterigmatocystis castanea* Patterson.[1]

[1]Bull. Torr. Bot. Club. **27**: 284. 1900.

Mr. Kearney's field notes are as follows:

"This manifests itself in a brownish discoloration of the seeds, which do not develop red color. In the later stages the seeds turn black and Mr. Drummond (of the U. S. Experimental Garden at Indio) says that finally when the fruit is opened a "puff of black soot" is released. Often individual black seeds are scattered through a mass of only slightly discolored ones. Sometimes certain segments of a fruit are affected while others appear healthy and even fairly colored. Rind and point of attachment often appear perfectly sound when the entire interior of the fruit is found to be badly infected. Fruits in the interior of the bush, well protected from the sun, are likely to be infected as much as those on the outside."

"I can only say that this condition was noticed at Indio last year

and appears to be very general there this year, at least 90% of the fruits of all varieties which I examined there last month having been more or less affected. At Sacaton, Arizona, on the other hand, only a small percentage of the varieties show this condition, the rest having the fruits perfectly sound At Indio it appears to have no connection with the sun scald which discolors the skins on exposed fruits, the internal rotting being as pronounced in fruits which have ripened in the dense shade in the center of the bushes as in those on the outside branches."

"I might add that I observed the same internal decay in pomegranate fruits growing on the ranch of F. C. Collins at San Antonio, Texas, so that it appears to be a pretty general condition in the Southwest."

The fact that the fungus generally shows no connection with the rind in the earlier stages of the disease leads one to think that the spores must gain entrance to the flower while the calyx is open. Subsequently the calyx closes and the fungus develops within the growing fruit. If this assumption be correct it is difficult to see how control can be effected. However, experiments will be made in the field during the coming season with a view to determining the exact time and manner of infection and this may lead to some method of control which is not now foreseen.

BUREAU OF PLANT INDUSTRY,
U. S. DEPT. OF AGRICULTURE,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES[1]

The Leaf Spot of the Pear. A reference to the consideration of this disease in two of our recent text-books on plant diseases where the causal organism is still referred to *Septoria pyricola* Desm. suggests the probability that the work of Klebahn was overlooked. (Duggar, B. M. Fungous Diseases of Plants, p. 358-60. 1909. Stevens, F. L., and Hall, J. G. Diseases of Economic Plants, p. 108, 1910.) *Septoria pyricola* has been definitely connected with an ascigerous form assigned to Mycosphaerella and according to Klebahn (Zeitschrift für Pflanzenkrankheiten. **18**: 5-17. 1908,) the name of the fungus now becomes *Mycosphaerella sentina* (Fckl.) Schrœter.

Inoculations upon the upper surfaces of pear leaves with ascospores expelled from perithecia of fallen leaves gave the characteristic Septoria spots in about two weeks, and at the end of four weeks mature spore-producing pycnidia were developed. Pure cultures from ascospores also developed pycnidia which produced the characteristic spores; further, the mycelium developed from Septoria conidia in cultures agreed perfectly with that originating from the ascospores. The ascus stage, however, was not obtained in cultures, and has been found only upon the fallen or dead leaves. The relation between the two stages of this fungus is quite similar to that which exists between the conidial and ascigerous stages of *Venturia pyrina*, and both of these troubles may be present upon pear leaves at the same time.

Saccardo and others claimed a connection between *Septoria pyricola* and another ascomycete, *Leptosphaeria lucilla* Sacc. This claim was not based upon experimental evidence, and the work of Klebahn shows that the supposition was incorrect.—F. D. HEALD

The staining of Bact. tumefaciens in tissue. It may be of interest to readers of this journal to know that we have finally succeeded in differentially staining the microorganism inside the cells of crown-gall, after many failures extending over a long period of time. The method which finally proved successful is a photographic one rather than a straight stain. We find that gold chlorid has the power of tinging the bacteria a deep blue-black without at the same time staining the protoplasm. Full details will be given in a forthcoming bulletin. It would seem

[1] Scientific notes not exceeding two hundred words in length, and news items of special interest to plant pathologists, will be regularly printed under this caption. Manuscripts may be sent directly to the editor in charge, Haven Metcalf, 1223 Vermont Avenue, Washington, D. C.

from preliminary studies that only a small proportion of the cells of the gall are actually occupied by this organism which we take to be *Bact. tumefaciens*. They occur in the form of rods two to three times as long as broad, in short chains, short filaments, and Y-shaped or variously-branched involution forms like those which commonly occur in old cultures of this organism.—ERWIN F. SMITH

Dr. C. N. Jensen, lately fellow in plant pathology in Cornell University, has become professor of botany and plant pathology in the Utah Agricultural College and Experiment Station. Mr. W. H. Rankin, also fellow in plant pathology in Cornell University, has been appointed forest pathologist to the New York State Conservation Commission. Mr. H. S. Fawcett, recently plant pathologist in the Florida Experiment Station, has been appointed plant pathologist to the California State Commission of Horticulture; and is succeeded in Florida by Mr. H. E. Stevens, formerly assistant in plant pathology in the Arkansas State University and Experiment Station. Mr. Fawcett's address is Whittier, Calif. Mr. H. P. Barss, lately instructor in botany and assistant plant pathologist in the Washington Agricultural College and Experiment Station, is appointed assistant professor of plant pathology in the Oregon Agricultural College and Experiment Station.

A bill has passed the Virginia legislature carrying an appropriation of five thousand dollars for the investigation and control of the chestnut bark disease. A similar bill in the Maryland legislature failed of passage.

Mark A. Carleton, for the past eighteen years in charge of Grain Investigations in the Bureau of Plant Industry, and well known as the introducer and propagator of Durum wheat and the Swedish Select oat, has resigned his present position to take charge of the work of the Pennsylvania Chestnut Tree Blight Commission. His address will be 1112 Morris Building, Philadelphia, Pa.

Mr. Paul J. Anderson, Fellow in the Department of Plant Pathology, Cornell University has resigned his position to take charge of field laboratory investigations of the chestnut canker disease for the Pennsylvania Chestnut Tree Blight Commission. His field station headquarters are at Tyrone, Pa.

REVIEWS

Some Recent Work on Peronosporales

The physiological problems concerning the infection of the host plant by these fungi have recently been under investigation by various mycologists and some noteworthy papers on the subject have appeared. These problems group themselves under three headings; (1) Conditions affecting germination of spores; (2) Conditions affecting host infection; (3) Relation of source of spores to infection, or the delimiting of the so-called "physiological species" which are present in the case of fungi having numerous hosts.

The most general in scope of the recent papers on these subjects is one by Melhus[1] on the white rust (*Albugo* or *Cystopus*) of crucifers. Extensive observations were made on the conditions affecting conidial germination and the consequent host infection. It was found that a copious germination of conidia could be secured by first subjecting to a temperature low enough to chill them. Germination was found to take place at temperatures ranging from slightly above zero C. to about 25° C., the maximum named by De Bary. While no optimum was established it appears to be about 10° C. It was also found that seasonal conditions affect conidial germination, those produced in spring and early summer germinating more rapidly than those produced late in the season. The conidia are rather highly resistant to cold since those taken from hosts killed by frost will germinate. Contrary to previous belief it was found that light and darkness has no appreciable effect on conidial germination, nor does atmospheric humidity exert any influence on the per cent of germination.

The conditions under which infection of the host takes place were studied carefully. It was found that higher percentages of infection were obtained when the plants were chilled during inoculation than when allowed to remain at greenhouse temperatures; the chilled plants showing as high as ninety-five per cent of infection while unchilled ones never showed over fifteen per cent. Earlier investigators have considered the cotyledons more favorable for infection than the leaves but the results of this investigation have shown no advantage in favor of either.

Experiments were also made to determine the existence of "physiological species." All the conidia used came from the radish (*Raphanus sativus*). These easily attacked *R. caudatus* and to a less extent *Brassica alba* and *B. oleracea* (cabbage) while numerous other hosts were unaffected.

[1] Melhus, I. E., Experiments on spore germination and infection in certain species of Oomycetes. Wisconsin Agr. Exp. Sta. Research Bul. 15: 25-91 *pl. 1-10.* 1911.

Of more restricted range, but none the less interesting, is a recent paper by Istvanffi and Palinkas,[1] who have studied the conditions affecting the infection of the grape by the downy mildew (*Rhysotheca viticola* (B. & C.) Wilson) which they refer to merely as Peronospora. Aside from the previous discovery by the senior author that the mycelium lives over winter in the host nothing definite was known concerning the overwintering and spring infection of this fungus. Indoor experiments showed that the incubation period covered about four or five days, the discolored spots and condia appearing by this time. The under side of the leaf also appeared to be more readily infected than the upper. Outdoor work showed certain points of divergence from these results probably due to the less constant atmospheric conditions. The incubation period was longer ranging from six to twelve days and averaging about ten. The greatest infection was obtained in cold damp weather although infection occurred in hot weather and even in times of low humidity. There was no advantage gained by wounding the epidermis. Experiments were also undertaken to determine the conditions under which the fruits become infected. The results were very similar to those obtained where leaves were used. The authors also found that spraying with copper sulphate when the yellow spots first appear prevented the development of conidia.

It was also found that atmospheric conditions exerted a marked influence upon the development of conidiophores and conidia. When the atmosphere is humid, large, much branched conidiophores are produced which bear from two hundred to four hundred conidia while in dry weather the conidiophores are much smaller and less branched, often bearing only ten to thirty conidia. "The more succulent the host, the thinner the epidermis, the greater the amount of water absorbed by the cells, the greater is the susceptibility of the host, as well as the danger of infection" and the more probable that an epidemic will occur.

About the same time Müller-Thurgau[2] was making observations and experiments along the same line with similar results. He also records observations on the presence or absence of oospores and specifies that young leaves and twigs are more susceptible than older ones.

These papers have given us an added insight into the problems of conidial germination, of host infection and of the conditions affecting spore formation which will prove most valuable in further study of the physiology of these fungi.—GUY WEST WILSON

[1] Istvanffi, Gy. von and Palinkas Gy. Infectionsversuche mit Peronospora. Centralb. f. Bakt. Abt. II, **32**: 551-564. 1912.

[2] Müller-Thurgau, H. Infektion der Weinrebe durch Plasmopara viticola. Centralb, f. Bakt. Abt. II. **29**: 683-695. 1911.

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